Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise

Harry R. Harding[1,2], Timothy A. C. Gordon[3,*], Rachel E. Hsuan[3], Alex C. E. Mackaness[1], Andrew N. Radford[1], Stephen D. Simpson[3]

1. School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ, UK
2. Marine Scotland Science, 375 Victoria Road, Aberdeen AB11 9DB, UK
3. Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK

* Corresponding author: Harry R. Harding, harry.harding@bristol.ac.uk

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Abstract

Anthropogenic noise can negatively impact many taxa worldwide. It is possible that in noisy, high-disturbance environments the range and severity of impacts could diminish over time, but the influence of previous disturbance remains untested in natural conditions. This study demonstrates effects of motorboat noise on the physiology of an endemic cichlid fish in Lake Malaŵi. Exposure to motorboats (driven 20–100 m from fish) and loudspeaker-playback of motorboat noise both elevated oxygen-consumption rate at a single lower-disturbance site, characterised by low historic and current motorboat activity. Repeating this assay at further lower-disturbance sites revealed a consistent effect of elevated oxygen consumption in response to motorboat disturbance. However, when similar trials were repeated at four higher-disturbance sites, no effect of motorboat exposure was detected. These results demonstrate that disturbance history can affect local population responses to noise. Action regarding noise pollution should consider the past, as well as the present, when planning for the future.

Introduction

Anthropogenic noise is present in many biomes across the planet, elevating overall acoustic energy and creating noises that are characteristically different from naturally occurring sounds [1–2]. Recent work has demonstrated that noise pollution can have a wide range of physiological and behavioural effects on many taxa (see [3,4] for reviews). Consequently, anthropogenic noise is considered a global pollutant that appears in international legislation, including the European Commission Marine Strategy Framework Directive and the US National Environment Policy Act.

To date, most studies investigating the consequences of anthropogenic noise for animals have used response means to test for overall impacts on a cohort of individuals, whilst largely ignoring the variation around the mean which may be driven by intrinsic characteristics or extrinsic

factors [5]. However, individual responses within a generation can be affected by prior experience; for example, organisms might exhibit altered tolerance through habituation, sensitisation or hearing threshold shifts, or emigrate because of past disturbances (see [6–8]). Furthermore, population responses may be altered over multiple generations through evolutionary adaptation. Experimental manipulations have shown that repeated exposure to anthropogenic noise can alter short-term responses in several species [8–9]. However, studies are lacking that explore how natural variation in responses are related to the long-term disturbance history of wild populations (see [7,10]).

Motorboat noise is increasing globally [11], and has a range of detrimental behavioural, physiological and fitness impacts on fishes [4,12] However, non-uniform distributions of boat use across space and time mean that fishes are exposed to varying levels of motorboat activity [1]. Here, we investigate how the impact of motorboat noise on a wild endemic cichlid in Lake Malaŵi is affected by variation in disturbance history. First, at a single site with low historic and current motorboat counts, we test a physiological response (oxygen consumption) to *in situ* exposures of both real motorboats driven around the testing site (hereafter referred to as 'motorboat disturbance') and loudspeaker playback of motorboat noise. The effect of motorboat disturbance on oxygen consumption was then tested at three further lower-disturbance sites. Finally, we used the same assay to test the response of fish to motorboat disturbance at four higher-disturbance sites.

Methods

Study system and sites

Work was conducted during April–July 2016 at Thumbi West Island, Lake Malaŵi (14° 01' S, 34° 49' E). Motorboat activity on Lake Malaŵi shows considerable spatial variability, with total usage likely to increase in the near future as a result of both human population increase [13] and development of fishing and tourism industries [14,15]. Adult males of the endemic cichlid *Cynotilapia zebroides*

(previously known as *C. afra* and *Microchromis zebroides*) were chosen as the study organism (see Supplementary Methods).

Sites were classified for disturbance levels based on analysis of both historic and current motorboat activity (full details in Supplementary Methods). Trip logs from the area's two local dive operators, which are representative of local near-shore motorboat traffic, were used to identify four lower-disturbance and four higher-disturbance sites. Boat counts of all vessel traffic confirmed that historic patterns matched current motorboating activity; at the time of the study, there were 10 times more boat-passes at higher-disturbance sites than lower-disturbance sites.

Acoustic stimuli

For playbacks, 10 independent 5-min underwater recordings of daytime ambient conditions (five different times of day) and motorboat noise (five different boats) were taken at the initial lower-disturbance site (see Supplementary Methods for details on playback-track creation and sound-level adjustment, which followed the methods in [16]). For the actual motorboat disturbance exposures, eight different boats were used across the eight sites (1–5 boats per site). Representative recordings of ambient conditions and motorboat noise were taken at each experimental site at the location of the fish during the trials. All recordings were analysed in both sound-pressure and particle-motion domains (Fig. S1; see Supplementary Methods for full details).

Identifying impacts of motorboat noise at a single lower-disturbance site

Oxygen-consumption rate is an emerging physiological tool for understanding likely impacts of anthropogenic pollutants on ecosystems [17]. The effect of motorboat noise on the oxygen-consumption rate of *C. zebroides* was tested *in situ* using an independent-measures experimental design. Oxygen-consumption rates were compared between fish exposed to either ambient

conditions, motorboat disturbance, or their playback equivalents. The complementary use of real motorboats and loudspeaker playback allowed both acoustic validity and isolation of motorboat noise as a stressor independent of visual cues and wake effects.

Fish were placed into an open-ended container for an acclimation period of 5 min before the container was sealed underwater in the lake and the sound treatment started; trials lasted for 30 min, with four fish run in parallel during each trial (full details in Supplementary Methods). Sealed containers were opaque, eliminating visual cues associated with exposure to motorboat disturbance. Water temperature and dissolved oxygen content in containers was measured at the start and end of the trial (Dissolved Oxygen and Temperature Meter HI 9164, Hanna Instruments Inc., Woonsocket, USA), and fish length and mass were recorded immediately after trials. Oxygen-content and mass data were used to calculate oxygen-consumption rates of fish over the trial period (mg $O_2/g_{fish}/h$). To assess the impact of motorboat noise, sound treatment (ambient sound or motorboat noise) and sound source (real sound or loudspeaker playback), and their interaction, were included as predictor variables in a two-way ANOVA.

Testing for effects of motorboat-disturbance history through multi-site comparisons

Having established qualitatively equivalent responses to motorboat disturbance and motorboat-noise playback (Fig. 1), motorboat disturbance was used exclusively for the multi-site comparisons to achieve acoustic validity. Assays were conducted at three additional lower-disturbance sites to investigate whether the oxygen-consumption response detected at the initial lower-disturbance site was consistently found. Assays were then conducted at four higher-disturbance sites to test whether the same response was apparent. Linear mixed models (LMMs) were used to control for the testing of multiple fish from the same sites.

Results

At the initial lower-disturbance site, there was a significant effect of sound treatment: fish exposed to motorboat noise exhibited higher oxygen-consumption rates than those exposed to ambient sound (two-way ANOVA: $F_{1,72} = 8.42$, $p = 0.005$; Fig. 1). However, there was no significant effect of sound source (real sound vs loudspeaker playback) ($F_{1,72} = 1.17$, $p = 0.28$), and no significant interaction between sound treatment and sound source ($F_{1,72} = 0.80$, $p = 0.37$).

The significant increase in oxygen-consumption rate in response to motorboat disturbance found at the initial lower-disturbance site was replicated when considering all four lower-disturbance sites (LMM: $\chi^2 = 9.239$, $df = 1$, $p = 0.002$, Intercept (ambient conditions) ± SE = 0.481 ± 0.008, Effect size ± SE = 0.036 ± 0.012; Fig. 2a). However, there was no significant effect of motorboat disturbance on oxygen-consumption rate at the four higher-disturbance sites ($\chi^2 = 0.786$, $df = 1$, $p = 0.375$, Intercept (ambient conditions) ± SE = 0.480 ± 0.010, Effect size ± SE = 0.012 ± 0.014; Fig. 2b). The variance associated with the random 'Site ID' term was less than 0.001 in both cases (Variance ± SD: Lower-disturbance <0.001 ± <0.001; Higher-disturbance <0.001 ± 0.008).

Discussion

This study used a rapid-assessment physiological assay to demonstrate that responses to motorboat noise in wild endemic cichlids in Lake Malaŵi were lower in areas with higher levels of motorboat disturbance. This represents novel *in situ* evidence to add to a small but increasing body of work examining intraspecific variation in organismal responses to noise. Such variation can occur due to both intrinsic characteristics (e.g. physiological body condition) and external factors (e.g. prior exposure) [8,10,18,19].

The ecological equivalence of all sites in this study (matched by depth, distance to shore, benthic substrate and water temperature) suggests that the observed difference in response to

noise was the result of differences in disturbance history. In contrast to previous laboratory-based extended exposures of fishes to noise [8], the lower response of fish at natural higher-disturbance sites could represent either acclimation within a generation (e.g. increased tolerance through shifts in hearing sensitivity thresholds, or a declining response from learning that the stimulus does not have any detrimental consequences [6–7]) and/or adaptation through selection over multiple generations (e.g. [20]). Indeed, tolerance may itself be a phenotypic trait subject to plasticity; for example, organisms with a higher tolerance of noise may have a selective advantage in high-disturbance areas through increased opportunities for foraging and mating [10,20]. Future work could use extended field-based manipulations of motorboat exposure within a generation to isolate disturbance history from any unmeasured and potentially confounding variables. This would facilitate further understanding of both the mechanistic drivers and the timescale over which such changes in tolerance develop [7].

Our study provides evidence from wild fishes that physiological responses to motorboats can be affected by existing variation in disturbance history, with the equivalent result shown in response to real motorboat and playbacks suggesting a strong influence of noise. Such intraspecific variation has implications for understanding and mitigating effects of noise on wildlife; for example, ecosystem impact assessments carried out in historically disturbed areas may represent underestimates of the threats posed to wider populations by novel sources of noise pollution. As such, we advocate more work on noise pollution that moves beyond consideration of whether there is an impact to investigations of what causes variation in responses.

Ethics: All work was performed in accordance with the Association for the Study of Animal Behaviour 'Guidelines for the treatment of animals in behavioural research and teaching', Malaŵian legal requirements, and institutional ethical approval (University of Bristol: UIN/13/036; University of Exeter: 2013/247), with permission from the Department of National Parks and Wildlife, Malaŵi.

Data accessibility: Data available from Dryad doi:10.5061/dryad.8ss7096

Authors' contributions: H.R.H., T.A.C.G., A.N.R. and S.D.S. conceived the research, designed experiments, conducted analyses and interpreted results; H.R.H., T.A.C.G., R.E.H. and A.C.E.M. conducted field-work; H.R.H. and T.A.C.G. prepared the initial manuscript and all authors contributed to subsequent revisions, gave final approval for publication, and agree to be held accountable for the work within the article.

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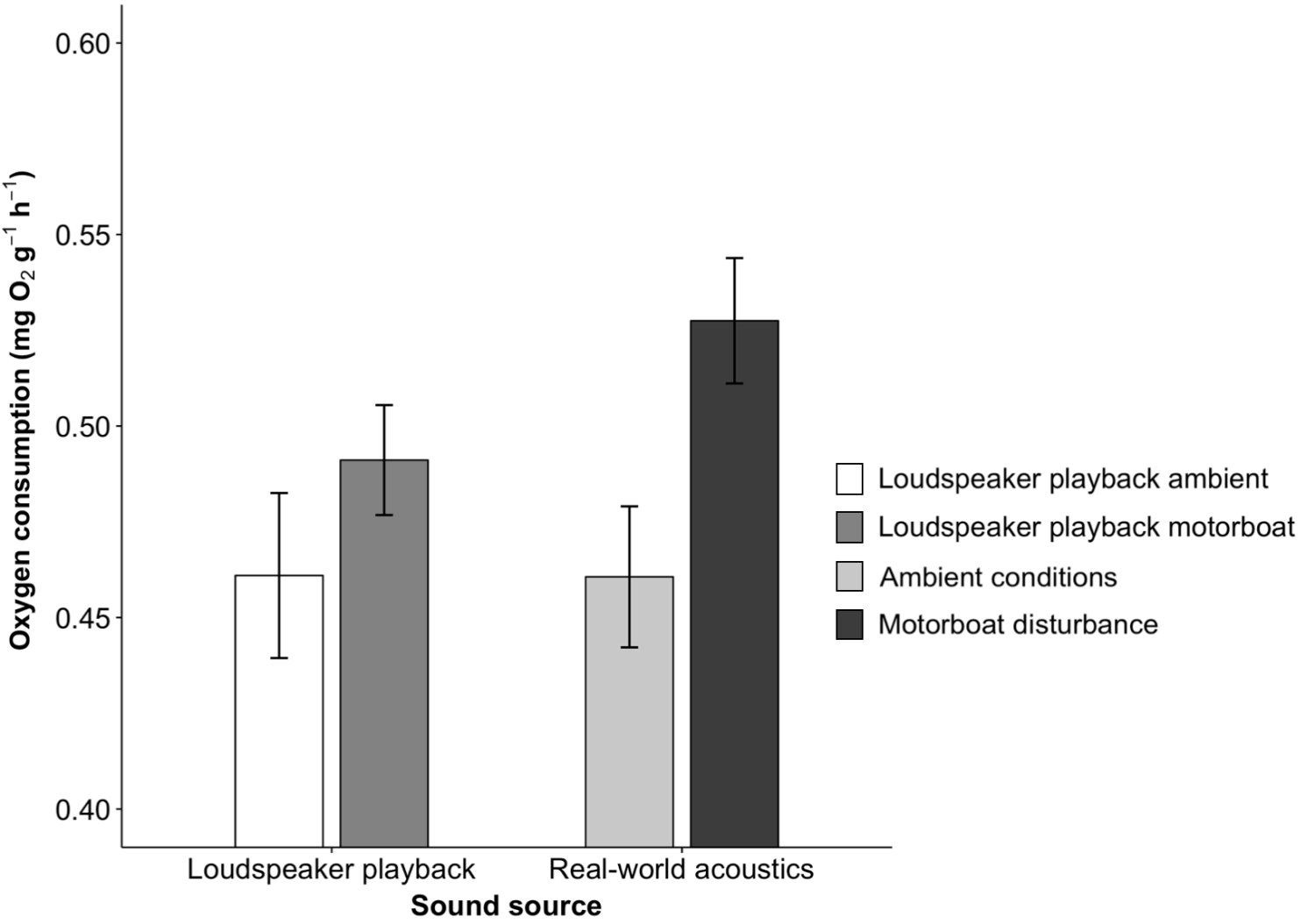

224 **Figure 1:** Mean ± SE oxygen consumption in *C. zebroides* exposed to playback of ambient sound (*n* =
225 19), playback of motorboat noise (*n* = 20), ambient conditions (*n* = 18) or motorboats (*n* = 19). Sound
226 treatment (ambient sound or motorboat noise) had a significant effect, but sound source (real sound
227 or loudspeaker playback) did not.

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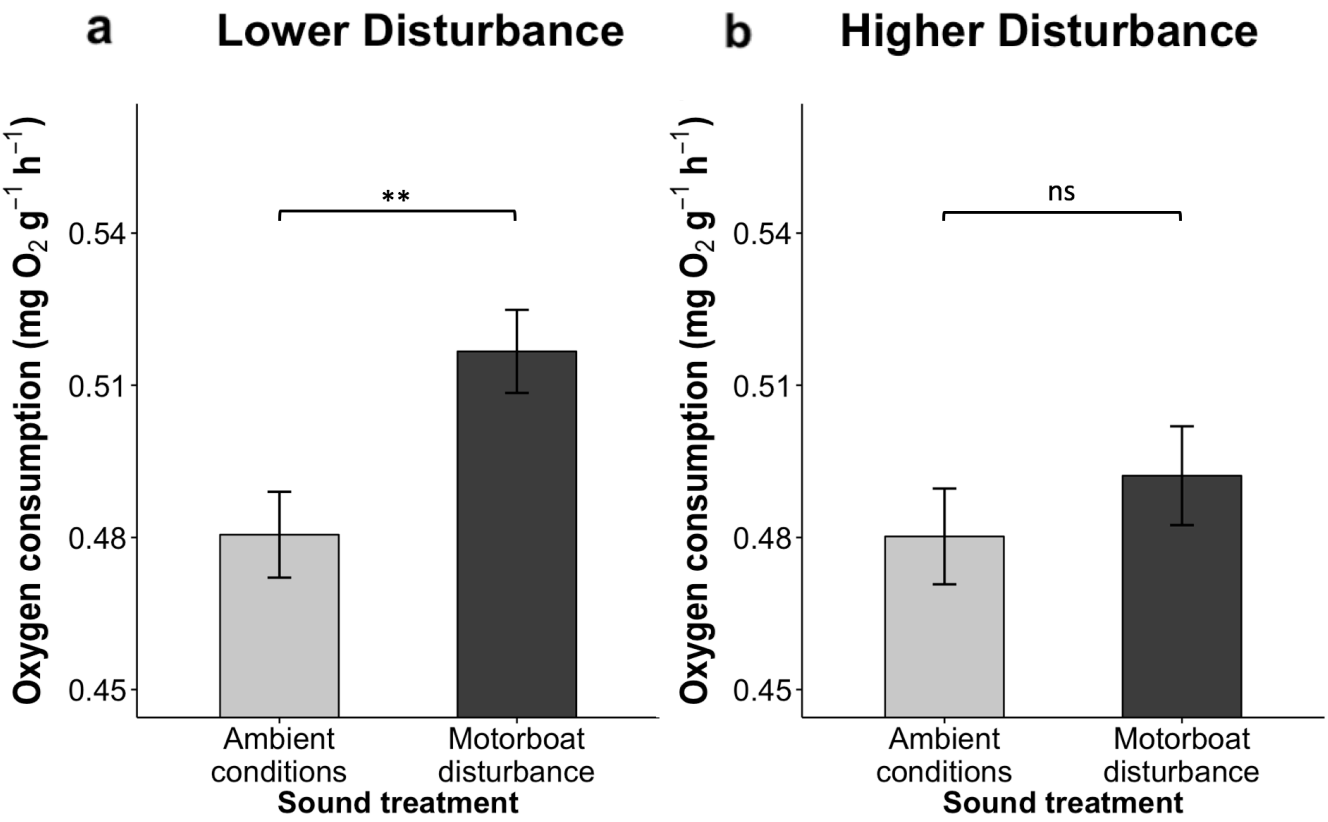

234 **Figure 2:** Mean ± SE oxygen consumption in *C. zebroides* exposed to ambient conditions or

235 motorboat disturbance in areas with (a) lower (ambient: *n* = 72; motorboats: *n* = 69) or (b) higher

236 (ambient: *n* = 71; motorboats: *n* = 70) current and historic levels of motorboat activity. ** *p* = <0.01;

237 ns denotes no significant difference.

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Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise

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Supplementary Methods

Study system and sites

The endemic cichlid *Cynotilapia zebroides* was chosen as the study organism based on: its abundance at the study site; its distinctive colouration allowing accurate identification despite the high species diversity and multiple intra-population colour polymorphisms present in the Lake Malaŵi species complex [S1, S2]; and previous evidence that acoustic cues play important roles in the ecology of similar cichlid species [S3, S4]. *C. zebroides* at Thumbi West Island represent a rapidly evolving population that are hybridising with the local *Metriaclima zebra* population [S2]; this is the case for all studied fish at all experimental sites.

Trip logs from both of the only two local dive operators (*Cape Maclear SCUBA* and *SCUBA Shack*), which represented all formally logged motorboat use in the area, were used to identify four lower-disturbance sites (no recorded visits in the last 3 years) and four higher-disturbance sites (visited in more than 8 of the last 12 months; mean ± SE, 51 ± 13 logged dives in 33 months) around the island. Combined visits to the four higher-disturbance sites comprised over 60% of total logged motorboating activity, and none of the four lower-disturbance sites ever appeared in either log. These logs provided a quantifiable and representative assessment of general patterns of local near-shore motorboat traffic by a much larger fleet of similar, unlogged vessels, since other motorboat users follow the leads of these dive operators when taking tourists to see fishes.

Counts of current activity includes all motorboat traffic in the area passing within 50 m of the selected sites (distance estimated visually; at least two counts per site with a total time of at least 4 h). Higher-disturbance sites (mean ± SE: 3.58 ± 0.82 boats/hr) experienced 10 times more

passes per hour on average than lower-disturbance sites (0.37 ± 0.19 boats/hr), and the passes were over twice as close to shore (mean ± SE, higher-disturbance sites: 19 ± 1.4 m; lower-disturbance sites: 45 ± 1.6 m). Boat counts were made between 10:30 and 17:00, with no difference in times between higher- and lower-disturbance sites. All selected sites were acoustically independent, shallow (2–5 m) coastal areas with a rocky benthic substrate, separated by a body of water with a minimum distance of 300 m and/or a prominent headland (see Fig. S2 for the locations of the eight sites around Thumbi Island West).

Acoustic stimuli

A single representative 5-min recording of the ambient conditions and motorboat noise used in the experiments was taken at each site. Recordings for playback experiments were taken in 2 m depth, 10 m from the shore. Ambient conditions had no boat traffic within 500 m. Motorboats were all of the type most commonly used in the area (7-m-long wooden hulls with 8–15 horsepower rear-mounted outboard engines), driven at various speeds 20–100 m from the recording equipment, as per [S5, S6].

Acoustic pressure was measured with a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.002–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS) connected to a digital recorder (PCM-M10, 48 kHz sampling rate, Sony Corporation, Tokyo, Japan). Particle motion was measured with a calibrated accelerometer (M20L; sensitivity following a curve over the frequency range 0–2 kHz; calibrated by manufacturers; Geospectrum Technologies, Dartmouth, Canada) connected to a digital 4-track recorder (Boss BR-800, 44.1 kHz sampling rate, Roland Corporation, Los Angeles, CA) and expressed as acceleration. Recording levels were calibrated using 1 kHz pure sine wave signals from a function generator with a measured voltage recorded in line on an oscilloscope. Sound-recording equipment was positioned 1 m above the lake bed attached to an

289 inflatable raft or a submerged stand to avoid unwanted noise from waves on the hull of a rigid boat.
290 The accelerometer was suspended by rope to reduce mechanical noise affecting the particle-motion
291 recordings.

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293 All tracks for playback experiments were created using Audacity 2.1.2 (www.audacityteam.org), and
294 adjusted to ensure that the root-mean-squared average amplitude levels (used as a measure of track
295 volume and analysed using SASLabPro v5.2.07; Avisoft Bioacoustics) received during trials were
296 equivalent to those received in original recordings. The sound system for playbacks consisted of a
297 loudspeaker (University Sound UW-30; maximal output 156 dB re 1 µPa at 1 m, frequency response
298 0.1–10 kHz; Lubell Labs, Columbus, OH) positioned 3 m from the trial, an amplifier (M033N, 18 W,
299 frequency response 0.04–20 kHz; Kemo Electronic GmbH, Germany), an MP3 player (BUSH, New
300 Mexico, USA), and a battery (12v 12Ah sealed lead-acid; CSB Co. Ltd, Vietnam). Representative
301 recordings of ambient sounds and motorboats were made at all sites for the multi-site comparison in
302 1.5–3 m depth, 6–13 m from shore.

303 All recordings were analysed in both sound-pressure and particle-motion domains using MATLAB
304 2013a (MathWorks Inc., PAMGuide & paPAM analysis packages [S13–S14]). Power spectral densities
305 were determined across the likely hearing range of cichlids; this was estimated as being the
306 frequency range 0–2 kHz, based on previous studies involving electrophysiological measurements of
307 hearing ability [S7–S9] and analysis of the frequency of courtship-associated cichlid vocalisations [S8,
308 S10, S11]. Playback using loudspeakers alters the characteristics of the original recordings, but
309 analyses of spectral content and sound levels showed that many of the characteristics of the original
310 recordings were retained in playback, and that these characteristics differed between playbacks of
311 ambient and motorboat noise (Fig. S1).

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Identifying impacts of motorboat noise at a single lower-disturbance site

During all experimental trials, fish were randomly allocated to different sound treatments to avoid selection bias, and the order of tracks or motorboats used was defined randomly within counterbalanced blocks. Treatment order was always alternated to avoid confounding effects of time of day or fish holding-time, and different motorboats and tracks were used in approximately equal numbers of trials.

At the start of each day, male *C. zebroides* were captured at the first lower-disturbance site by snorkelers using a 10 x 1 m barrier net in 2–5 m depth, within a 200 m stretch of coastline. Captured individuals were held during transport and for storage before testing in an opaque barrel containing 20 L of regularly flushed lake water. The testing location was within 500 m of capture, transport was by paddling, and the holding barrel was kept on the shore to ensure acoustic isolation from the experimental trials. Fish were released unharmed to their natal sites at the end of each day, and capture was always over 50 m away from capture sites on previous days; due to the high site-fidelity of territorial Lake Malaŵi cichlids [S12], this avoided the chance of re-testing fish on consecutive days.

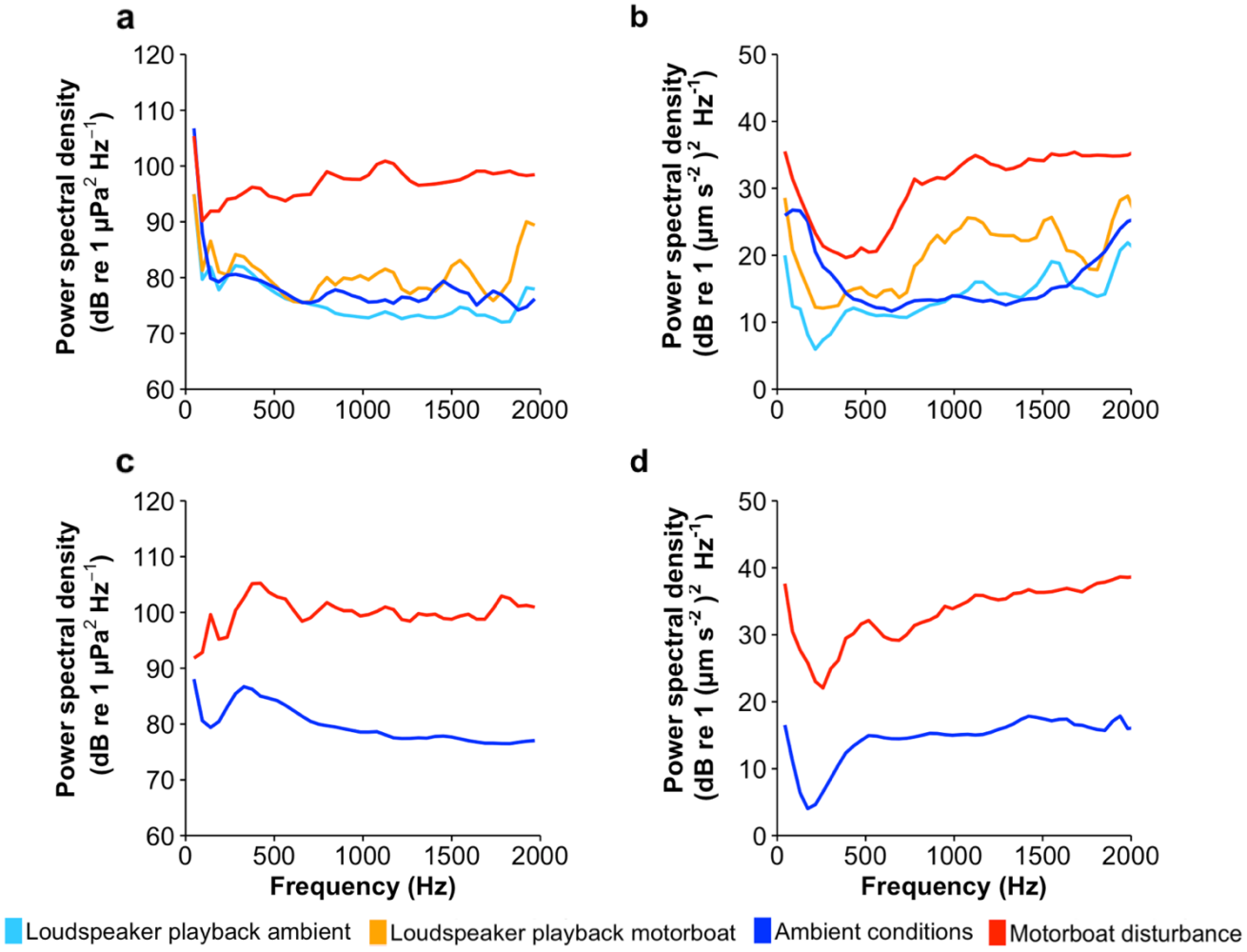
To measure oxygen-consumption rate, individual fish (mean ± SE, length: 8.51 ± 0.04 cm; mass: 9.24 ± 0.15 g) were placed in a section of open PVC piping (9.2 x 3.8 cm) with mesh netting over each end to restrict movement, inside a sealed polyethylene terephthalate (PET) container (13.7 x 6.2 cm; 412 ml volume) suspended from a submerged stand 1.5–3 m below the surface of the lake. Acoustic transparency was predicted to be high due to similar acoustic impedance values for water (1.5 MRayls) and PET (1.76 MRayls). Following a 5-min acclimation period, fish were sealed in containers for a 30-min sound-exposure period. Containers were then brought to the surface and fish were removed. All capture, handling and measurement methods were identical for noise-exposed and control fish, facilitating valid comparisons of relative differences in oxygen-consumption rate. There was no significant difference in either length (one-way ANOVA: $F_{3,70} = 0.38$,

p = 0.77) or mass (Kruskal-Wallis test: H_3 = 0.56, p = 0.91) of fish allocated to different sound treatments.

Testing for effects of motorboat-disturbance history through multi-site comparisons

Experimental protocols followed those for the initial lower-disturbance site. All experimental sites consisted of a rocky benthic substrate and were characteristically similar in depth (2–6 m), distance to shore (6–13 m) and mean water temperature (23.8–25.3°C). There was no significant difference in trial temperature between treatments in either the higher-disturbance (two-sample t-test: t = -0.42, n_{amb} = 72, n_{boat} = 69, p = 0.67) or lower-disturbance (n_{amb} = 71, n_{boat} = 70, t = -0.74, p = 0.46) sites. There was no significant difference in either mass (Mann-Whitney U-tests, higher-disturbance: W = 2401, n_{amb} = 72, n_{boat} = 69, p = 0.73; lower-disturbance W = 2663, n_{amb} = 71, n_{boat} = 70, p = 0.46) or length (two-sample t-tests, higher-disturbance: t = -0.33, n_{amb} = 68, n_{boat} = 64, p = 0.74; lower-disturbance: t = 0.76, n_{amb} = 70, n_{boat} = 65, p = 0.45) of fish allocated to different sound treatments at the lower- and higher-disturbance sites.

Linear mixed models (LMMs) were used to analyses datasets from lower-disturbance and higher-disturbance sites. In both LMMs, sound treatment (ambient or motorboat) was used as a fixed term, site ID (1–4) was included as a random term, and significant effects of sound treatment were confirmed by comparisons with a null model. Visual examination of residual plots never revealed any obvious deviations from homoscedasticity or normality.



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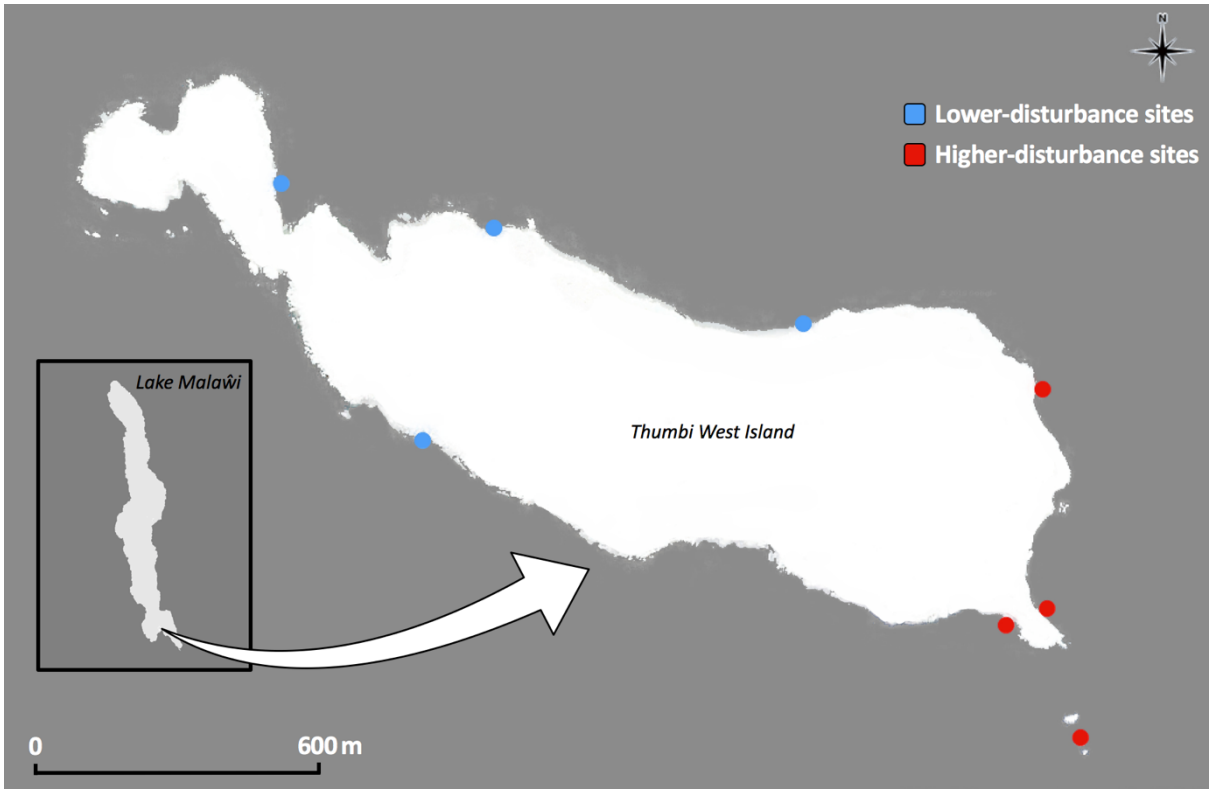
358 **Supplementary Figure S1:** Acoustic analyses of experimental conditions. (a & b) Mean spectral
359 content of combined illustrative recordings of original ambient conditions and motorboat noise, and
360 playback of those recordings in both (a) pressure and (b) particle-motion domains, recorded at the
361 initial single lower-disturbance test site. Root-mean-squared sound-pressure levels (0–2 kHz) and
362 range are: 115.1, 113.3–116.4 (Ambient playback); 117.9, 112.4–121.2 (Motorboat playback); 125.1,
363 115.4–128.0 (Ambient); and 132.2, 127.5–134.9 (Motorboat disturbance). Particle-acceleration
364 levels (0–2kHz) and range (given in dB re (1µm/s^2)) are 48.7, 44.6–52.9 (Ambient playback); 55.9,
365 43.8–62.4 (Motorboat playback); 53.6, 46.7–55.6 (Ambient); and 67.1, 64.1–69.0 (Motorboat
366 disturbance). (c & d) Mean spectral content of combined illustrative recordings of original ambient
367 conditions and motorboat noise taken at each subsequent site, in both (c) pressure and (d) particle-
368 motion domains. Fast Fourier Transform (FFT) analysis of 0–2 kHz, spectrum level units averaged

from 30 s recordings, Hamming evaluation window, FFT size = 1024. Root-mean-squared sound-pressure levels (0–2 kHz) and range (given in dB re 1 µPa) are: 115.5, 109.8–120.6 (Ambient); and 135.0, 120.8–142.4 (Motorboat disturbance). Particle-acceleration levels (0–2kHz) and range (given in dB re (1µm/s^2)) are: 49.1, 46.6–52.2 (Ambient); and 69.3, 62.9–76.8 (Motorboat disturbance).



Supplementary Figure S2: Image showing the location of the eight sites around *Thumbi West Island* (14° 1’ 14” S, 34° 49’ 9” E). All experimental sites were matched for benthic substrate (rocky bottom), depth (2–6 m), distance to shore (6–13 m) and mean water temperature (23.8–25.3°C).

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